3229

Potentially Infectious Agents Associated with Shearling Bedpads

I. Effect of Laundering with Detergent-Disinfectant Combinations on Polio and Vaccinia Viruses

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Glutaraldehyde-tanned woolskin pads which are used for the prevention of decubitus ulcers in bed patients were experimentally contaminated with polio or vaccinia viruses. Two methods of exposure, direct contact and aerosol, were used in separate experiments. Attempts were made to remove or inactivate these virus contaminants by laundering the woolskins in a quaternary ammonium disinfectant, a phenolic disinfectant, or alkalinized glutaraldehyde, in combination with an anionic detergent or a nonionic detergent. The effect of a commercial detergent-sanitizer was also studied. The virus titers were significantly reduced in all experiments, but only laundering in glutaraldehyde in combination with either detergent lowered the vaccinia virus titers to below detectable limits. High concentrations of glutaraldehyde altered the texture of the wool and leather apparently by precipitating a component of the detergent onto the fibers. In all the poliovirus experiments, the virus was still detectable on either or both the wool and the leather of the pads after laundering. The rinse water from each experiment was tested for the presence of virus. No vaccinia virus was recovered, but poliovirus was demonstrated in titers up to 10^3 cell culture 50% infectious doses.

A special glutaraldehyde-tanned shearling (woolskin) has been developed as a nursing aid in the prevention and cure of decubitus ulcers (bedsores) in hospital patients through its use as a bedpad (6). When such material is used for these purposes, methods must be devised whereby it can be cleaned and rendered free of pathogens so that it can be reused, particularly since studies have indicated that both viruses and bacteria can persist on items such as fabrics for long periods of time (2, 12). Since no standard method for this sanitization is known, the present studies were carried out in an attempt to develop such a method. In these studies, representative pathogenic bacteria and viruses were used. These included *Staphylococcus aureus*, *Pseudomonas aeruginosa*, poliovirus, and vaccinia virus. This report describes the studies carried out with the vaccinia and the polio viruses.

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MATERIALS AND METHODS

Woolskins. Shearling medical pads (woolskins) were obtained from A. C. Lawrence Leather Co., Peabody, Mass. They were tanned with a combination of glutaraldehyde and basic chromium sulfate by a process based on reported U.S. Department of Agriculture research (6).

Viruses. The MEF-1 strain of type 2 poliovirus and the Lederle chorioallantoic strain of vaccinia virus, obtained from Parke, Davis and Co., were used in these studies. The viruses were prepared in human epidermoid carcinoma of the larynx (HEp-2) cells (9).

Detergents. The following detergents were used. (i) A detergent-sanitizer, containing the lauryl pyridinium salt of 5-chloro-2-mercaptobenzothiazole (0.20%), 2-2-methylenebis (3,4,6-trichlorophenol; 0.05%), brominated isomers of salicylanilide "typically composed of" 95% 3,5,4′-tribromosalicylanilide, and 5% "related isomers" (0.35%), was manufactured by Swift Chemical Co., Chicago, Ill., and was used in wash water at concentrations of 665 and 1,330 ppm (3/4 lb/100 lb of laundry). (ii) Anionic detergent, a sodium alkylaryl polyether sulfate provided in an

aqueous dispersion by the Rohm and Haas Co., Philadelphia, Pa., was used in wash water at a concentration of 265 ppm. (iii) Nonionic detergent, an alkylaryl polyether alcohol provided in liquid form by the Rohm and Haas Co. as an industrial detergent and emulsifier, was used in wash water at a concentration of 265 ppm.

Disinfectants. The following disinfectants were used for these experiments. (i) Quaternary ammonium disinfectant, consisting of *n*-alkyl (C_{14}, C_{12}, C_{16}) dimethyl benzyl ammonium chloride (80%), ethyl alcohol, and water, was obtained from the Rohm and Haas Co. and used in wash water at concentrations of 60 and 120 ppm. (ii) Phenolic disinfectant was ortho-benzyl-para-chlorophenol ($C_{13}H_{11}OCl$), provided in solid (flake) form by the Monsanto Co., St. Louis, Mo., and was used in wash water at concentrations of 1,000 and 2,000 ppm. (iii) A 50% solution of glutaraldehyde was obtained from the Fisher Chemical Co., Fair Lawn, N.J., for these studies. Solutions of 0.5, 1, 2, and 4%, buffered to *p*H 9, were used in the vaccinia virus studies. For the poliovirus work, 2 and 4% solutions were used. Alkaline solutions were utilized, since it has been reported (1) that glutaraldehyde is most effective as a disinfectant at a basic *p*H level.

A sour was used in rinse water at a concentration of 1,272 ppm to render a less alkaline *p*H to the laundered material. This material (obtained from Swift Chemical Co., Chicago, Ill.) was composed of 99.30% ammonium silicofluoride, 0.05% moisture (at 37 C), 0.08% free acid (H_2SiF_6), 0.04% insoluble matter, and trace quantities of iron sulfate and phosphate. The sour was added to the rinse water for a 3-min rinse after the wash and the first 3-min rinse cycle.

Washing machine. The washing machine used was an automatic, top-loading, agitator-type (Lady Kenmore, Sears, Roebuck and Co., Chicago, Ill.). The maximum capacity of the machine was approximately 68.4 liters (18 gal). The recommended maximum washing load was approximately 6.3 kg (14 lb). After each complete use of the machine, the interior was flushed with water at a temperature of ca. 64 C; preliminary tests with *S. aureus*, *P. aeruginosa*, and *Serratia marcescens* indicated that this exposure to hot water eliminated all detectable organisms from the interior of the machine. The exterior of the machine was swabbed with 70% ethyl alcohol.

Methods of exposure to virus. Direct contact and aerosol methods of exposure were used. In the direct-contact method, each shearling swatch was held by sterile forceps, and 1.0 ml of virus suspension was pipetted onto the wool side. For the aerosol method, two 25-ml bottles were filled with virus suspension. The bottles were connected to model 152 DeVilbiss atomizers set up facing each other, 20 inches (50 cm) apart, in a molded plastic isolator. The aerosol was produced by the atomizers under 13 psi of nitrogen gas and was allowed to settle on the swatches for 1 hr. The swatches were pinned to a whole shearling pad in a uniform pattern, with both the swatches and the pad placed with the wool side up. The aerosol produced contained particles of which 95% were from 0.27 to 60 μm in diameter, at a density of 2.1×10^5 particles per liter, as determined by an aerosol photometer (17).

Method for determining effectiveness of wash procedures. Shearling swatches (5.08 cm diameter) were cut with a mechanized die. The swatches and whole shearlings were sterilized with ethylene oxide (STERI-VAC Sterilizer, 3-M Co., St. Paul Minn.), as described previously (14). Five sterile swatches were pinned to a sterile whole shearling pad. The shearling with the attached swatches, an additional whole shearling, and five additional swatches were contaminated with the virus by one of the exposure methods. The five additional swatches were reserved as unlaundered controls to determine the initial concentration of pathogen. The second shearling was used to provide balance in the washing machine and to provide additional virus to the total, so that any reduction of the virus which was brought about by laundering would not be a result of simple dilution in the wash water. The two shearlings and the five attached swatches were laundered with a test detergent and disinfectant, or detergent only, at a temperature of 50 $\pm$ 6 C for 10 min. This laundering was followed by a 3-min rinse in water at a temperature of 39 $\pm$ 6 C. The sour was then added, and an additional 3-min rinse at the decreased *p*H was carried out at the same temperature. A 6-min spin-dry cycle was used to remove the major portion of the water from the pad. The wash agitation speed was as follows: 70 agitations/min for 4 min, 48 agitations/min for 4 min, and then 70 agitations/min for 2 min. A 10-ml amount of the second rinse water was removed for assay of virus content. Immediately after the spin-dry cycle, the swatches were removed and the wool was mechanically separated from the leather. The wool was placed in a 40-ml homogenizer cup (Ivan Sorvall, Inc., Norwalk, Conn.). The leather was cut into small pieces and placed in another homogenizer cup. A 25-ml amount of Eagle basal medium (4) supplemented with 5% agamma calf serum and 0.5% chick embryo extract was added to each cup. The material was macerated by running the homogenizer, placed in an ice bath, at maximum speed for 30 sec. The eluate was removed and centrifuged at low speed, and the virus titer of the supernatant fluid was determined by assay in HEp-2 cells grown in vinyl plastic panels (10). Each sample was run in quintuplicate panel cups, and the mean virus titers of the eluate were determined from these five titers. All virus titers were expressed as cell culture 50% infectious doses/ml of eluate ($CCID_{50}$/ml).

Each test included toxicity controls, in which the eluates from macerated sterile laundered wool or leather were tested for cytotoxic effects in the same cell culture system. Eluates from sterile unlaundered wool and leather were similarly tested.

The five virus-exposed swatches (virus controls) which were not laundered were tested for virus at the same time as the test swatches. To evaluate the effect of a particular laundering process, the mean virus titer from the wool and leather of the five test swatches was compared to the mean titer of the similar material from the virus control swatches.

The sample of rinse water removed for virus assay

was diluted in serial 10-fold dilutions and pipetted directly into quintuplicate panel cups.

RESULTS

The results of an experiment typical of those carried out in this study are shown in Table 1. The experiments in which vaccinia virus was placed on the woolskins by direct contact are summarized in Table 2. Laundering in water reduced the virus titer 4.5 $\log_{10}$ on the wool, but the virus titers on the leather were decreased only approximately 1 $\log_{10}$. Laundering in either detergent had little or no more effect than laundering in water only. Glutaraldehyde, at the lowest concentration employed and used with either detergent, reduced the vaccinia virus titers on both wool and leather to below detectable levels. No other detergent-disinfectant combination produced complete elimination of demonstrable virus. No virus was recovered from the rinse water in any experiment.

The experiments in which vaccinia virus was placed on the woolskins in an aerosol are summarized in Table 3. Often less virus was recovered from the swatches exposed to aerosolized virus than was recovered from the direct-contact virus-exposed swatches, but the virus titer reductions brought about by laundering in detergent or disinfectants (or both) were comparable to those observed in the direct-contact experiments.

In the direct-contact exposure poliovirus experiments (Table 4), laundering in water reduced the virus titers approximately 2 $\log_{10}$ on the swatches. In no experiment were the poliovirus titers reduced to below detectable limits on both the wool and the leather of a group of test swatches, although the use of glutaraldehyde with either detergent markedly affected the titers on both the wool and the leather. Laundering in glutaraldehyde or phenolic disinfectant and anionic detergent resulted in no virus being recovered from the rinse water, but in the majority of the experiments, virus in concentrations as high as 2.8 $\log_{10}$ was demonstrated in the rinse water.

The aerosol exposure poliovirus experiments

TABLE 1. *Effect of laundering with nonionic detergent and 4% glutaraldehyde on the titer of vaccinia virus placed on woolskins by direct contact*

Description of sample	Swatch no.	Cytotoxicity[a]	Virus titer[b] ($CCID_{50}$/ml)	Mean virus titer ($CCID_{50}$/ml)	Virus titer reduction
Virus control[c]—wool	1	pt $10^{-0.9}$	$10^{5.9}$		
	2	pt $10^{-0.4}$	$10^{5.9}$		
	3	pt $10^{-0.9}$	$10^{5.6}$	$10^{6.0}$	
	4	pt $10^{-0.4}$	$10^{5.9}$		
	5	pt $10^{-0.4}$	$10^{6.2}$		
Test[d]—wool.	6	pt $10^{-2.4}$	$<10^{2.4}$		$>10^{3.9}$
	7	pt $10^{-1.4}$	$<10^{1.4}$		
	8	pt $10^{-1.4}$	$<10^{1.4}$	$<10^{2.1}$	
	9	pt $10^{-1.4}$	$<10^{1.4}$		
	10	pt $10^{-2.4}$	$<10^{2.4}$		
Virus control[c]—leather	11	pt $10^{-1.9}$	$10^{4.9}$		
	12	pt $10^{-1.9}$	$10^{5.0}$		
	13	pt $10^{-1.4}$	$10^{5.2}$	$10^{4.9}$	
	14	pt $10^{-1.9}$	$10^{4.2}$		
	15	pt $10^{-1.9}$	$10^{4.9}$		
Test[d]—leather	16	pt $10^{-1.9}$	$<10^{1.9}$		$>10^{3.0}$
	17	pt $10^{-1.9}$	$<10^{1.9}$		
	18	pt $10^{-1.9}$	$<10^{1.9}$	$10^{1.9}$	
	19	pt $10^{-1.9}$	$<10^{1.9}$		
	20	pt $10^{-1.9}$	$<10^{1.9}$		
Rinse water		$10^{-2.4}$	$<10^{2.4}$	$<10^{2.4}$	

[a] Expressed as the maximum dilution of eluate or rinse water which was toxic to HEp-2 cells. pt = Partially toxic, but viral CPE still distinguishable.

[b] Each swatch eluate was tested in quintuplicate; therefore, the titers shown are the mean of each quintuplicate test.

[c] Exposed to virus but not processed through laundering or rinsing.

[d] Exposed to virus and processed through washing and rinsing as described.

(Table 5) yielded results similar to those obtained from the direct-contact experiments. As was the case in the vaccinia virus studies, less virus (one to two $\log_{10}$) was recovered from the aerosol-exposed control swatches than was obtained from the direct-contact virus-exposed swatches, but the titers were sufficiently high to demonstrate significant titer reductions. Launder-

TABLE 2. *Effect of laundering with detergents and with detergents and disinfectants on the titer of vaccinia virus placed on woolskins by direct contact*

Detergent	Disinfectant		Virus titer reduction ($\log_{10}$)		Recoverable virus in rinse water ($\log_{10}$)
	Type	Concn	Wool	Leather	
None	None		4.5	1.3	<0.9
Nonionic	None		3.3	1.1	<1.4
Anionic	None		3.8	2.5	<1.4
Nonionic	Quaternary	60 ppm	4.0	1.7	<1.4
Nonionic	Quaternary	120 ppm	5.1	2.7	<0.9
Nonionic	Phenolic	1,000 ppm	3.9	2.3	<0.9
Nonionic	Phenolic	2,000 ppm	>3.0	2.5	<0.9
Nonionic	Glutaraldehyde	0.5%	>4.9	>3.2	<0.9
Nonionic	Glutaraldehyde	1.0%	>4.5	>3.8	<1.4
Nonionic	Glutaraldehyde	2.0%	>3.1	>0.6	<1.4
Nonionic	Glutaraldehyde	4.0%	>3.9	>3.0	<2.4
Anionic	Quaternary	60 ppm	3.4	2.0	<0.9
Anionic	Quaternary	120 ppm	>4.1	3.0	<0.9
Anionic	Phenolic	1,000 ppm	4.0	1.9	<0.9
Anionic	Phenolic	2,000 ppm	4.0	2.6	<0.9
Anionic	Glutaraldehyde	0.5%	>4.8	>3.1	<1.4
Anionic	Glutaraldehyde	1.0%	>4.9	>2.9	<1.4
Anionic	Glutaraldehyde	2.0%	>4.8	>3.2	<2.4
Anionic	Glutaraldehyde	4.0%	>3.9	>3.3	<2.4
Combination[a]		655 ppm	4.8	>3.4	<0.9
Combination[a]		1,310 ppm	5.2	3.0	<0.9

[a] Detergent-sanitizer.

TABLE 3. *Effect of laundering with detergents and with detergents and disinfectants on the titer of vaccinia virus placed on woolskins by aerosol*

Detergent	Disinfectant		Virus titer reduction ($\log_{10}$)		Recoverable virus in rinse water ($\log_{10}$)
	Type	Concn	Wool	Leather	
Nonionic	None		2.9	1.2	<0.9
Anionic	None		3.7	1.7	<0.9
Nonionic	Quaternary	60 ppm	3.9	1.7	<0.9
Nonionic	Quaternary	120 ppm	4.6	>1.7	<0.9
Nonionic	Phenolic	1,000 ppm	>3.3	2.1	<0.9
Nonionic	Phenolic	2,000 ppm	>3.0	2.3	<0.9
Nonionic	Glutaraldehyde	0.5%	>3.4	>2.1	<0.9
Nonionic	Glutaraldehyde	1.0%	>3.8	>1.5	<1.4
Nonionic	Glutaraldehyde	2.0%	>2.5	>0.6	<1.4
Nonionic	Glutaraldehyde	4.0%	>2.2	>1.1	<2.4
Anionic	Quaternary	60 ppm	>4.0	1.1	<0.9
Anionic	Quaternary	120 ppm	>3.4	>1.4	<0.9
Anionic	Phenolic	1,000 ppm	>3.7	1.7	<0.9
Anionic	Phenolic	2,000 ppm	>2.9	1.1	<0.9
Anionic	Glutaraldehyde	0.5%	>3.5	>2.1	<1.4
Anionic	Glutaraldehyde	1.0%	>4.4	>2.5	<1.4
Anionic	Glutaraldehyde	2.0%	>2.6	>0.6	<2.4
Anionic	Glutaraldehyde	4.0%	>2.3	>0.3	<2.4
Combination[a]		655 ppm	5.0	>2.1	<0.9
Combination[a]		1,310 ppm	3.5	>1.6	<0.9

[a] Detergent-sanitizer.

ing in detergent or in detergent and disinfectant affected the virus titers essentially the same among this group as described in the above direct-contact studies. Virus was again often recovered in significant titers from the rinse water.

The wool, leather, and the rinse water exhibited varying degrees of residual cytotoxicity, depending on the type of detergent or disinfectant (or both) used in the laundering process. The eluates from those swatches laundered with only the detergents were essentially nontoxic, but those materials laundered in glutaraldehyde and in the phenolic disinfectants were toxic in eluate dilutions up to 10^{-3}.

Variation among the test samples in a given direct contact virus-exposed group was usually less than ± 1 $\log_{10}$. The aerosol-exposed swatches often varied slightly more, but this variation was

TABLE 4. *Effect of laundering with detergents and with detergents and disinfectants on the titer of poliovirus placed on woolskins by direct contact*

Detergent	Disinfectant		Virus titer reduction ($\log_{10}$)		Recoverable virus in rinse water ($\log_{10}$)
	Type	Concn	Wool	Leather	
None	None		2.6	1.7	1.9
Nonionic	None		3.1	1.1	0.9
Anionic	None		3.8	1.5	<0.9
Nonionic	Quaternary	60 ppm	3.2	1.4	1.2
Nonionic	Quaternary	120 ppm	2.8	2.6	2.8
Nonionic	Phenolic	1,000 ppm	2.4	1.5	1.9
Nonionic	Phenolic	2,000 ppm	4.0	1.8	1.2
Nonionic	Glutaraldehyde	2%	5.3	4.8	<1.4
Nonionic	Glutaraldehyde	4%	5.3	4.2	2.4
Anionic	Quaternary	60 ppm	2.8	1.3	2.2
Anionic	Quaternary	120 ppm	2.7	1.9	3.0
Anionic	Phenolic	1,000 ppm	3.9	2.7	<0.9
Anionic	Phenolic	2,000 ppm	2.5	1.5	<1.4
Anionic	Glutaraldehyde	2%	4.1	>5.0	<1.4
Anionic	Glutaraldehyde	4%	5.3	5.3	<2.4
Combination[a]		655 ppm	4.2	2.4	<0.9
Combination[a]		1,310 ppm	3.7	2.0	1.6

[a] Detergent-sanitizer.

TABLE 5. *Effect of laundering with detergents and with detergents and disinfectants on the titer of poliovirus placed on woolskins by aerosol*

Detergent	Disinfectant		Virus titer reduction ($\log_{10}$)		Recoverable virus in rinse water ($\log_{10}$)
	Type	Concn	Wool	Leather	
Nonionic	None		4.7	2.6	1.1
Anionic	None		5.2	3.3	<0.9
Nonionic	Quaternary	60 ppm	2.5	1.3	1.2
Nonionic	Quaternary	120 ppm	2.1	1.1	2.9
Nonionic	Phenolic	1,000 ppm	3.9	1.5	1.9
Nonionic	Phenolic	2,000 ppm	3.9	2.5	0.9
Nonionic	Glutaraldehyde	2%	3.9	3.4	<2.4
Nonionic	Glutaraldehyde	4%	3.9	2.8	<2.4
Anionic	Quaternary	60 ppm	3.3	0.7	1.3
Anionic	Quaternary	120 ppm	2.0	0.0	2.9
Anionic	Phenolic	1,000 ppm	3.5	0.5	<0.9
Anionic	Phenolic	2,000 ppm	4.1	1.7	<0.9
Anionic	Glutaraldehyde	2%	4.0	3.2	<1.4
Anionic	Glutaraldehyde	4%	3.9	2.7	<2.4
Combination[a]		655 ppm	3.1	1.7	<0.9
Combination[a]		1,310 ppm	3.1	0.1	1.6

[a] Detergent-sanitizer.

alleviated by randomizing the samples and using five in each group to determine a mean virus titer.

Glutaraldehyde, the most effective disinfectant used against both vaccinia and poliovirus in these experiments, was objectionable to a degree because of its strong odor at the highest concentrations. Also, it altered the texture of the wool and leather, apparently from the precipitation of either anionic or nonionic detergents. Reducing the concentration of this disinfectant to 0.5% virtually eliminated these two problems, although the woolskins were still toxic to the cultured cells used in the study after processing through it.

DISCUSSION

It was apparent that laundering of virus-containing woolskins in either water alone, water and detergent, or in a combination of water, detergent, and disinfectant markedly lowered the titer of detectable virus, although the actual effects on the viruses cannot be ascertained in studies such as this. Large quantities of virus were used in each laundering process in an attempt to overcome the problem of elution masking any viral inactivation which may have occurred. No vaccinia virus was recoverable from the rinse water in any experiment, whereas relatively high titers of poliovirus were demonstrated in the rinse water. This difference suggests that the vaccinia virus may be inactivated in the laundering process but that the poliovirus may merely have been eluted from the swatches. Since virus was recoverable from the majority of the laundered samples, it is apparent that any inactivation that occurred was not complete in those cases. Glutaraldehyde, apparently the most effective disinfectant used in these studies, is known to have virucidal activity against both polio and vaccinia virus, as well as against other viruses (5, 7, 11, 15, 16). Quaternary ammonium compounds have been demonstrated to inactivate vaccinia virus but are ineffective against poliovirus (7, 13). Klein and DeForest reported ortho-phenyl-phenol to be active against vaccinia virus and ineffective against poliovirus (7), but we know of no published data concerning the virucidal effectiveness of ortho-benzyl-para-chlorophenol, which was used in the present studies. The manufacturer's sales handbook (Santophen 1, p. 4.5–4.9, Monsanto Chemical Co., St. Louis, Mo.) for this product describes experiments in which it completely inactivated herpes simplex, vaccinia, adeno type 2, and Asian influenza viruses. No data were cited concerning the inactivation of poliovirus. The detergent-sanitizer combination used in these studies contained a thiazole, a cholorophenol, and a bromosalicylanilide. No data are available concerning the virucidal activity of such a combination; previous studies carried out in our laboratory indicate the bromosalicylanilides to have little activity against polio or vaccinia viruses (13).

Since laundering in water only of the virus-contaminated shearlings resulted in noticeable virus reductions, we may conclude that the observed reductions in virus titers could have been partially due to certain physical factors such as temperature and the immersion in water resulting from processing the woolskins through the washing machines. The *p*H may not have been an important factor in this inactivation, since it was relatively similar in all experiments. The final rinse water, after addition of sour, had a *p*H of approximately 6; the wool and the leather of the laundered samples were slightly basic, with a *p*H of approximately 7.5.

The method of exposure of the woolskins to the viruses had no significant effect on the rate of viral reduction, although the samples exposed to aerosolized virus usually received slightly less virus than those exposed by direct contact. The leather particularly had lower titers of either virus when exposed to the virus aerosols. Such a result would be expected, since the thick covering of wool would prevent penetration to the leather.

The cytotoxicity noted in certain of these experiments may be an important problem when actual in-use applications of these laundering methods are considered, since such cytotoxicity apparently reflects residual amounts of disinfectant or detergent remaining on the swatches. Such residual chemicals may cause skin irritation, thus actually reversing the woolskin advantage of preventing decubitus ulcers. The *p*H was apparently not the primary cause of the cytotoxicity noted, since in almost all experiments the wool and leather eluates had a relatively neutral *p*H.

The problem of method limit has been recognized as inherent in any experiment of a virucidal nature (8), and was a factor in the present studies. Thus, in order to determine whether virus was present on a test sample, the samples had to be macerated in a certain quantity of eluting fluid, and then the eluate had to be added to cells which have an additional amount of culture medium. These two steps resulted in a mandatory dilution of the original virus to a total of $10^{0.9}$ $CCID_{50}$/ml. Since the virus titers were reduced to this limit in few cases, method limit was not a major problem in these studies.

Since poliovirus in titers up to 10^3 $CCID_{50}$ was recovered from the rinse water, the likelihood exists that this virus could be transmitted to non-contaminated materials laundered with the virus-containing woolskins. This potential for contamination of other materials is considered much

less for the vaccinia virus, since none was recovered from the rinse water in these experiments.

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